IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KUBOTA=9

In re Application of:	)	Art Unit:
Michio KUBOTA et al	)	Examiner:
I.A. No.: PCT/JP01/06412	)	
I.A. Date: July 7, 2001	)	
	)	Washington, D.C.
U.S. Appln. No.:	)	
Date Nationalized: Even Date Herewith	)	Confirmation No.
For: α-ISOMALTOSYLGLUCOSACCHARIDE-FORMING ENZYME, PROCESS AND USES OF THE SAME	)	April 1, 2002

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to any action on the merits, please amend as follows:

IN THE CLAIMS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached version is captioned "**Version with Markings to Show Changes Made**".

Please amend the claims as shown below:

5. (Amended) The α-isomaltosylglucosaccharide-forming enzyme of claim 1, which comprises one or more amino acid

sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NOs:5 to 7, SEQ ID NOs:11 to 14, and SEQ ID NO:18.

6. (Amended) The α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 or 4, which is stabilized and/or activated by Ca^{2+} and Mn^{2+}.

7. (Amended) The α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1, 2, 4 or 5, which is a purified or crude enzyme.

8. (Amended) A process for producing the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1, 2, 4 or 5, which comprises:

culturing in a nutrient culture medium a microorganism capable of producing said enzyme;

and collecting said enzyme from the resulting culture.

12. (Amended) A method of α-glucosyl-transferring reaction, which comprises a step of contacting the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1, 2, 4 or 5 with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end.

14. (Amended) A method for forming α-isomaltosyl-glucosaccharide, which comprises a step of contacting the α-

isomaltosylglucosaccharide-transferring enzyme of any one of claims 1, 2, 4 or 5 with a solution, comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, to effect α-glucosyl-transferring reaction.

16. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by contacting both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1, 2, 4 or 5 and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor.

18. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by:

contacting both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1, 2, 4 or 5 and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end to obtain a solution comprising said cyclotetrasaccharide along with other saccharide(s); and

subjecting said solution to column chromatography using a strong-acid cation exchange resin,

wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor.

19. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 18, which contains at least 30% (w/w), on a dry solid basis, of the cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

20. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 18, which is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystalline powder, or crystalline solid.

22. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 20, wherein said crystal is prepared by crystallizing in an aqueous solution without using any organic solvent.

23. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by contacting both the α-isomaltosylglucosaccharide-transferring enzyme of claim 1, and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor,

wherein said saccharide comprising cyclotetrasaccharide is one which comprises cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} and one or more saccharides selected from the group consisting of glucose, maltose, and a saccharide having a glucose polymerization degree

of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the non-reducing end.

24. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by culturing a microorganism in a nutrient culture medium comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, said microorganism being capable of producing both the α-isomaltosylglucosaccharide-forming enzyme as claimed in claim 1 and an α-isomaltosyl-transferring enzyme which specifically hydrolyzes the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety and which transfers the released α-isomaltosyl moiety to an acceptor.

25. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 24, wherein said saccharide having a glucose polymerization degree of at least two is one selected from the group consisting of maltooligosaccharides, maltodextrins, amylodextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

29. (Amended) The cyclotetrasaccharide or the saccharide composition as claimed in claim 24, which is obtainable by:

contacting the resulting culture or the saccharide obtainable from the culture with one or more enzymes selected from the group consisting of α-amylase, β-amylase, glucoamylase, and α-glucosidase; and

treating the resultant mixture with one or more purification methods selected from the group consisting of decoloration, desalting, fractionation by column chromatography, separation with a membrane, fermentation with a microorganism, and alkaline treatment.

30. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 24, wherein said α-isomaltosyl-transferring enzyme has the following physicochemical properties:

(1) Action

Forming a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} from a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the

α-1,4 glucosidic linkage other than the above linkage;

(2) Molecular weight

Having a molecular weight of about 82,000 to about 136,000 daltons when determined on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE);

(3) Isoelectric point (pI)

Having a pI of about 3.7 to about 8.3 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 45_C to about 50_C when incubated at a pH of 6.0 for 30 min;

(5) Optimum pH

Having an optimum pH of about 5.5 to about 6.5 when incubated at 35_C for 30 min;

(6) Thermal stability

Having a thermostable range at temperatures of about 45_C or lower when incubated at a pH of 6.0 for 60 min;

(7) pH Stability

Having a stable pH range at about 3.6 to about 10.0 when incubated at 4_C for 24 hours.

31. (Amended) A process for producing a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which comprises a step of contacting with a solution of gelatinized and/or liquefied starch both the α-isomaltosylglucosaccharide-transferring enzyme of claim 1 and an α-isomaltosyl-transferring enzyme, which specifically hydrolyzes the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme and which transfers the released α-isomaltosyl moiety to an acceptor.

34. (Amended) The process of claim 32, which further contains one or more purification methods selected from the group consisting of decoloration, desalting, fractionation by column chromatography, separation with a membrane, fermentation treatment using microorganisms, and decomposition by an alkaline treatment.

35. (Amended) The process of claim 31, which contains at least 30% (w/w), on a dry solid basis, of a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

36. (Amended) The process of claim 35, wherein said cyclotetrasaccharide or said saccharide composition comprising the same is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystalline powder, or crystalline solid.

38. (Amended) The process of claim 31, wherein said α-isomaltosyl-transferring enzyme has the following physicochemical properties:

(1) Action

Forming a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} from a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the above linkage;

(2) Molecular weight

Having a molecular weight of about 82,000 to about 136,000 daltons when determined on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE);

(3) Isoelectric point (pI)

Having a pI of about 3.7 to about 8.3 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 45_C to about 50_C when incubated at a pH of 6.0 for 30 min;

(5) Optimum pH

Having an optimum pH of about 5.5 to about 6.5 when incubated at 35_C for 30 min;

(6) Thermal stability

Having a thermostable range at temperatures of about 45_C or lower when incubated at a pH of 6.0 for 60 min;

(7) pH Stability

Having a stable pH range at about 3.6 to about 10.0 when incubated at 4_C for 24 hours.

39. (Amended) A composition comprising the cyclotetrasaccharide of claim 16, having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}, or a saccharide composition comprising the same.

41. (Amended) The composition of claim 40, which is in the form of a food product, cosmetic, or pharmaceutical.

42. (Amended) The composition of claim 40, which comprises at least 0.1% (w/w), on a dry solid basis, of the cyclotetrasaccharide.

REMARKS

The above amendments are to avoid improper multi-dependencies. Even though some multiply dependent claims remain, applicants believe that all claims in the application are now properly dependent, i.e. each multiply dependent claim depends from a claim which is not multiply dependent on another claim.

Applicants respectfully await the results of a first examination on the merits.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By _____
Sheridan Neimark
Registration No. 20,520

SN:jaa
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
F:\,s\suma\kubota9\pto\Amd Pre mltidpcy.doc

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5. (Amended) The α-isomaltosylglucosaccharide-forming enzyme of ~~any one of claims 1 to 4~~claim 1, which comprises one or more amino acid sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NOs:5 to 7, SEQ ID NOs:11 to 14, and SEQ ID NO:18.

6. (Amended) The α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 ~~to 5~~or 4, which is stabilized and/or activated by Ca^{2+} and Mn^{2+}.

7. (Amended) The α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1, 2, 4 or 5 ~~to 6~~, which is a purified or crude enzyme.

8. (Amended) A process for producing the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1, 2, 4 or 5 ~~to 7~~, which comprises ~~the steps of~~:

culturing in a nutrient culture medium a microorganism capable of producing ~~the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 6~~said enzyme;

and collecting said enzyme from the resulting culture ~~the α-isomaltosylglucosaccharide-forming enzyme of any one of claims 1 to 7~~.

12. (Amended) A method of α-glucosyl-transferring reaction, which comprises a step of contacting the α-isomaltosylglucosaccharide-transferring enzyme of any one of

claims 1, 2, 4 or 5 ~~to 7~~ with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end.

14. (Amended) A method for forming α-isomaltosyl-glucosaccharide, which comprises a step of contacting the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1~~—~~, 2, 4 or 5~~to 7~~ with a solution, comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, to effect α-glucosyl-transferring reaction.

16. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by contacting both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1, 2, 4 or 5 ~~to 7~~ and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the

α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor.

18. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by:

contacting both the α-isomaltosylglucosaccharide-transferring enzyme of any one of claims 1, 2, 4 or ~~5 to 7~~ and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end to obtain a solution comprising said cyclotetrasaccharide along with other saccharide(s); and

subjecting said solution to column chromatography using a strong-acid cation exchange resin,

wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor.

19. (Amended) The cyclotetrasaccharide or the saccharide composition of ~~any one of claims 16 to~~claim 18, which contains at least 30% (w/w), on a dry solid basis, of the

cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

20. (Amended) The cyclotetrasaccharide or the saccharide composition of ~~any one of claims 16 to 19~~claim 18, which is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystalline powder, or crystalline solid.

22. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 20 ~~or 21~~, wherein said crystal is prepared by crystallizing in an aqueous solution without using any organic solvent.

23. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by contacting both the α-isomaltosylglucosaccharide-transferring enzyme of claim 1, and an α-isomaltosyl-transferring enzyme with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, wherein said α-isomaltosyl-transferring enzyme specifically hydrolyzing the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide which is formed by the action of the α-

isomaltosylglucosaccharide-transferring enzyme, and transferring the released α-isomaltosyl moiety to an acceptor,

~~The cyclotetrasaccharide or the saccharide composition of any one of claims 16 to 22,~~ wherein said saccharide comprising cyclotetrasaccharide is one which comprises cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} and one or more saccharides selected from the group consisting of glucose, maltose, and a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the non-reducing end.

24. (Amended) A cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which is obtainable by culturing a microorganism in a nutrient culture medium comprising a saccharide having a glucose polymerization degree of at least two and having the α-1,4 glucosidic linkage as a linkage at the non-reducing end, said microorganism being capable of producing both the α-isomaltosylglucosaccharide-forming enzyme as claimed in ~~any one of claims 1 to 7~~claim 1 and an α-isomaltosyl-transferring enzyme which specifically hydrolyzes the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety and

which transfers the released α-isomaltosyl moiety to an acceptor.

25. (Amended) The cyclotetrasaccharide or the saccharide composition of claim 24, wherein said saccharide having a glucose polymerization degree of at least two is one selected from the group consisting of maltooligosaccharides, maltodextrins, amylodextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

29. (Amended) The cyclotetrasaccharide or the saccharide composition as claimed in ~~any one of claims 24 to 28~~claim 24, which is obtainable by:

contacting the resulting culture or the saccharide obtainable from the culture with one or more enzymes selected from the group consisting of α-amylase, β-amylase, glucoamylase, and α-glucosidase; and

treating the resultant mixture with one or more purification methods selected from the group consisting of decoloration, desalting, fractionation by column chromatography, separation with a membrane, fermentation with a microorganism, and alkaline treatment.

30. (Amended) The cyclotetrasaccharide or the saccharide composition of claim ~~16, 18 or~~ 24, wherein said α-isomaltosyl-transferring enzyme has the following physicochemical properties:

(1) Action

Forming a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} from a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the α-1,4 glucosidic linkage other than the above linkage;

(2) Molecular weight

Having a molecular weight of about 82,000 to about 136,000 daltons when determined on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE);

(3) Isoelectric point (pI)

Having a pI of about 3.7 to about 8.3 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 45_C to about 50_C when incubated at a pH of 6.0 for 30 min;

(5) Optimum pH

Having an optimum pH of about 5.5 to about 6.5 when incubated at 35_C for 30 min;

(6) Thermal stability

Having a thermostable range at temperatures of about 45_C or lower when incubated at a pH of 6.0 for 60 min;

(7) pH Stability

Having a stable pH range at about 3.6 to about 10.0 when incubated at 4_C for 24 hours.

31. (Amended) A process for producing a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} or a saccharide composition comprising the same, which comprises a step of contacting with a solution of gelatinized and/or liquefied starch both the α-isomaltosylglucosaccharide-transferring enzyme of ~~any one of claims 1 to 7~~claim 1 and an α-isomaltosyl-transferring enzyme, which specifically hydrolyzes the linkage between an α-isomaltosyl moiety and the resting glucosylsaccharide moiety of an α-isomaltosylglucosaccharide formed by the action of the α-isomaltosylglucosaccharide-transferring enzyme and which transfers the released α-isomaltosyl moiety to an acceptor.

34. (amended) The process of claim ~~31, 32 or 33~~, which further contains one or more purification methods selected from the group consisting of decoloration, desalting, fractionation by column chromatography, separation with a

membrane, fermentation treatment using microorganisms, and decomposition by an alkaline treatment.

35. (Amended) The process of ~~any one of claims~~claim 31~~ to 34~~, which contains at least 30% (w/w), on a dry solid basis, of a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}.

36. (Amended) The process of ~~any one of claims 31 to~~claim 35, wherein said cyclotetrasaccharide or said saccharide composition comprising the same is in the form of a syrup, massecuite, amorphous powder, amorphous solid, crystalline powder, or crystalline solid.

38. (amended) The process of claim 31~~ or 33~~, wherein said α-isomaltosyl-transferring enzyme has the following physicochemical properties:

(1) Action

Forming a cyclotetrasaccharide having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→} from a saccharide having a glucose polymerization degree of at least three and having both the α-1,6 glucosidic linkage as a linkage at the non-reducing end and the

α-1,4 glucosidic linkage other than the above linkage;

(2) Molecular weight

Having a molecular weight of about 82,000 to about 136,000 daltons when determined on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE);

(3) Isoelectric point (pI)

Having a pI of about 3.7 to about 8.3 when determined on isoelectrophoresis using ampholine;

(4) Optimum temperature

Having an optimum temperature of about 45_C to about 50_C when incubated at a pH of 6.0 for 30 min;

(5) Optimum pH

Having an optimum pH of about 5.5 to about 6.5 when incubated at 35_C for 30 min;

(6) Thermal stability

Having a thermostable range at temperatures of about 45_C or lower when incubated at a pH of 6.0 for 60 min;

(7) pH Stability

Having a stable pH range at about 3.6 to about 10.0 when incubated at 4_C for 24 hours.

39. (Amended) A composition comprising the cyclotetrasaccharide of ~~any one of claims~~claim 16 ~~to 30~~, having the structure of cyclo{→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→6)-α-D-glucopyranosyl-(1→3)-α-D-glucopyranosyl-(1→}, or a saccharide composition comprising the same.

41. (amended) The composition of claim ~~39 or~~ 40, which is in the form of a food product, cosmetic, or pharmaceutical.

42. (amended) The composition of claim ~~39 or~~ 40, which comprises at least 0.1% (w/w), on a dry solid basis, of the cyclotetrasaccharide.